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Effect of Chlorhexidine loading on Shear Bond Strength and Surface Free Energy of Acrylic Reline Resins after Thermal Ageing

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Resumo

A reabilitação com próteses removíveis é uma das hipóteses de tratamento para a perda dentária total ou parcial, permitindo o restabelecimento da função, dimensão vertical, estética, fonética, entre outros.

A perda de peças dentárias está associada a uma reabsorção óssea crónica e progressiva, revelando-se mais acentuada na mandíbula. Desta forma, o rebasamento das próteses permite uma readaptação aos tecidos de suporte e o prolongamento da sua utilização, evitando uma antecipada substituição por uma prótese nova.

Os materiais de rebasamento podem ser de consistência macia, tal como os acondicionadores de tecidos, ou de consistência rígida, como as resinas acrílicas de rebasamento. A desvantagem dos primeiros está associada à sua rápida deterioração, sendo apenas viáveis num intervalo de semanas, tendo de ser substituídos com bastante regularidade. Contrariamente, as resinas acrílicas de rebasamento apresentam-se como uma solução viável mais duradoura.

As resinas acrílicas são constituídas por polímeros obtidos através de uma reação de polimerização. Durante esta, nem todo o monómero é convertido, o que significa que haverá a libertação de substâncias potencialmente citotóxicas para a cavidade oral e possibilitar-se-á a formação de porosidades na resina. Estas porosidades permitem a colonização de variados microrganismos, como é o caso da *Candida albicans*, que é atualmente aceite ser um dos principais microrganismos responsáveis pelo desenvolvimento de Estomatite Protética.

A Estomatite Protética é uma patologia com frequência elevada em pacientes idosos, imunocomprometidos, com fraca higiene oral ou que não realizam o descanso noturno da prótese. Os antifúngicos tópicos e sistémicos surgem como opção de tratamento mais frequente, contudo, estão associados a reincidências, já que não vão diretamente à origem do problema: a colonização das próteses por microrganismos.

A literatura científica indica que a clorexidina, antissético com ação antibacteriana e antifúngica, mais frequentemente usado em Medicina Dentária sob a forma de bochecho a 0,2%, possui capacidade de suprimir a aderência de *Candida albicans* à mucosa. Quando este

fármaco é incorporado em resinas, mostrou que possui ação antimicrobiana superior aos antifúngicos, como o fluconazol, e que apresenta inibição da aderência da *Candida albicans*. No entanto, há que considerar que esta incorporação de fármaco e respetiva libertação pode comprometer as propriedades físicas das resinas e o seu desempenho.

Considerando o facto de que o conhecimento científico atual não contempla o comportamento das resinas incorporadas com clorexidina, quando sujeitas a processos de biodegradação na cavidade oral, como o envelhecimento térmico, revela-se importante centrarem-se estudos nesta matéria.

Desta forma, o objetivo deste estudo foi avaliar o efeito da incorporação de diferentes concentrações de clorexidina, na resistência ao corte e energia de superfície, de três resinas acrílicas de rebasamento, Kooliner, Ufi Gel Hard e Probase Cold; após serem sujeitas a um processo de envelhecimento.

Para testar a resistência ao corte, foram preparados cento e vinte espécimes (12×10×6 mm) de resina termopolimerizável de base de prótese, os quais foram submetidos a 2500 ciclos de termociclagem (alternadamente submersos a 5 e 55ºC). Foram posteriormente reduzidos a 3mm de espessura e divididos por três grupos, cada um correspondente a uma resina. Para a resina Kooliner (n=10) foram atribuídos 40 espécimes, para a Ufi Gel Hard (n=10) foram distribuídos 50 espécimes, restando 30 espécimes para a resina Probase Cold (n=10). O número de grupos foi escolhido com base nas concentrações de clorhexidina incorporada em cada material, que tiveram por base resultados de estudos anteriores (Kooliner - 0%; 2,5%; 5% e 7,5%; Ufi Gel Hard – 0%; 2,5%; 5%; 7,5% e 10%; Probase Cold - 0%; 2,5% e 5%). Procedeu-se ao rebasamento das resinas da base de acordo com a concentração selecionada para cada grupo e submeteram-se os espécimes rebasados a 1000 ciclos de termociclagem. Seguidamente, os espécimes foram sujeitos a uma máquina de testes universal Instron e efetuou-se o teste de resistência ao corte. As superfícies previamente aderidas foram observadas num estereomicroscópio para que fosse permitida a classificação do tipo de falha: adesiva, quando não há vestígios de resina de rebasamento na resina da base da prótese ou vice-versa; mista, quando há vestígios de resina de rebasamento na resina da base da prótese ou vice-versa; coesiva, quando a superfície da base da prótese está preenchida por resina de rebasamento.

Para o teste de energia de superfície, recorreram-se a moldes de aço (125×25×1 mm) e, com base nas diferentes concentrações selecionadas para cada material (Kooliner – 0%; 2,5% e 5%; Ufi Gel Hard – 0%; 5% e 7,5%; Probase Cold – 0%; 2,5% e 5%), foram elaborados sessenta e três espécimes (25×16×1 mm) das três resinas acrílicas de rebasamento (n=7). As concentrações escolhidas tiveram também por base estudos anteriores. Os espécimes foram submetidos a termociclagem de 1000 ciclos e posteriormente testados. Recorrendo a um tensiómetro de *Kruss* e através da técnica da placa de *Wilhelmy*, foram obtidos os ângulos de contacto em água e em propilenoglicol, sendo determinada posteriormente a energia de superfície pelo método de *Wu*.

Os resultados foram analisados estatisticamente com recurso a testes não paramétricos, pelo método de *Kruskal-Wallis*, com subsequentes comparações de *Mann-Whitney* e correção de *Bonferroni*. Considerou-se nível de significância de 5% em todos os testes.

Quanto à resistência ao corte, não se verificaram diferenças estatisticamente significativas nos grupos experimentais de Kooliner e Ufi Gel Hard quando comparados com o controlo. Relativamente ao Probase Cold, o grupo de 5% apresentou valores inferiores de resistência ao corte comparativamente com o grupo controlo.

Respeitante à análise do tipo de falha dentro dos grupos de cada resina, registaram-se 100% falhas adesivas em todos os grupos experimentais de Kooliner; quanto aos grupos de Ufi Gel Hard: verificaram-se 100% falhas adesivas nos grupos controlo e com 2,5% de clorexidina; 70% falhas adesivas e 30% falhas mistas no grupo de 5% de clorexidina; 60% falhas adesivas e 40% falhas mistas nos grupos de 7,5% e 10% de clorexidina; finalmente, nos grupos de Probase Cold registaram-se: 30% falhas adesivas, 30% falhas mistas e 40% falhas coesivas no grupo controlo; 50% falhas adesivas, 40% falhas mistas e 10% falhas coesivas no grupo de 2,5% clorhexidina; e 60% falhas adesivas e 40% falhas mistas no grupo de 5%.

No que respeita a energia de superfície, não foram encontradas diferenças estatisticamente significativas em qualquer um dos grupos de cada material.

Em conclusão, a incorporação de clorexidina, após um processo de envelhecimento não parece afetar a resistência ao corte nos grupos de Kooliner e Ufi Gel Hard e parece influenciar negativamente os espécimes de Probase; não parece afetar a energia de superfície das três resinas acrílicas de rebasamento em estudo.

Considerando as limitações deste estudo, revelam-se necessários mais estudos concernentes às propriedades mecânicas destes materiais que utilizem outros métodos de avaliação da adesão entre resinas, com amostras que tenham um maior número de espécimes e que avaliem diferentes propriedades mecânicas, e que possam explicar também a deformação plástica do material Kooliner, averiguando se é justificável a utilização de outras resinas de rebasamento que lhe sejam superiores.

Palavras-chave: Resinas acrílicas de rebasamento; Clorexidina; Envelhecimento térmico; Resistência ao corte; Energia de Superfície.

Abstract

Loading acrylic reline resins with chlorhexidine has been proposed as an alternative treatment for Denture Stomatitis.

The main goal of this study was to evaluate the influence of loading three acrylic reline resins, namely, Kooliner, Ufi Gel Hard and Probase Cold, with different concentrations of chlorhexidine (CHX) in terms of their shear bond strength and surface free energy after undergoing a thermal ageing process.

Shear bond strength test was conducted resorting to a universal testing machine Instron, with 1kN load cell and crosshead speed of 1mm/min, with specimens of acrylic reline resins loaded with specific proportions of CHX linked to the denture base resin (n=10), after submitted to a thermal process that simulates ageing inside the oral cavity.

Surface free energy was assessed resorting to specimens of acrylic reline resins loaded with specific percentages of CHX (n=7). Specimens were subjected to water and 1,2-propanediol to assess contact angles by the Wilhelmy plate technique, which allows the estimation of surface free energy values through the Wu method.

Results were submitted to nonparametric tests according to the Kruskal-Wallis method, followed by multiple comparisons using Mann-Whitney tests with Bonferroni corrections, being considered the 5% level of significance (α=0.05). No differences in shear bond strength were observed when experimental Kooliner and Ufi Gel Hard groups where compared to control. Yet, 5% CHX Probase Cold group presented lower values than the control. In surface free energy test, statistical differences were not observed between groups of each material.

In conclusion, after a thermal ageing procedure, loading CHX had no influence on shear bond strength of Kooliner and Ufi Gel Hard groups but a negative influence on Probase Cold on 5% CHX specimens. Loading chlorhexidine did not affected the surface free energy of all acrylic reline resins.

Keywords: Acrylic reline resins, Chlorhexidine; Thermal ageing; Shear bond strength, Surface free energy.

1. Introduction

Tooth loss is associated to a decrease of integrity of the masticatory system, which will promote negative functional, esthetic and psychological consequences (Verma *et al.*, 2017). With the world population growing and living longer associated to the current economic status, rehabilitation with removable prostheses will remain one of the preferred options. This kind of rehabilitation allows the reestablishment of function, vertical dimension, improves aesthetics and speech (Owen *et al*., 2000; Dhir *et al*., 2007).

One of the consequences of tooth loss is the bone resorption (Pedlar *et al*., 2007). After extraction, the alveolar crest will suffer a progressive and irreversible resorption, which become stable in the maxilla after three years and follows a continuous resorption of 0,4mm per year in the jaw, for a period of fifteen years (Atwood *et al*., 1971; Talgreen, 1972; Reis *et al.*, 2006; Devlin *et al*., 2012). With this physiological event, removable prosthesis tends to misfit and the result is trauma in the underlying mucosa and the rejection of the denture (Rahn *et al.,* 2009; Devlin *et al*., 2012).

To overcome this bone loss and allow the use of the prostheses in their full function, soft materials like tissue conditioners can be applied. Although, they reveal to loss their mechanical and physical properties with time, become difficult to clean and need replacement at short intervals (Devlin *et al*., 2012). The other alternative lays on a relining procedure with hard acrylic reline resins. The relining procedure allows a resurface of the base of an existing denture with new denture base material (Rahn *et al*., 2009). Relining materials can be divided into chairside relining materials, which means that the relining is directly performed inside the mouth of the patient; or laboratory relining materials, an indirect method (Rahn *et al*., 2009). These relining materials, alike to the denture base, are acrylic resins, polymeric biomaterials composed by chains of monomers, where a maximum conversation of monomer is necessary to have a good polymerization, a minimal release of potential cytotoxic substances into the oral cavity and the lowest emergence of porosities that allow the colonization by microorganisms, that promote the development of oral diseases, like Denture Stomatitis. (Radnai *et al*., 2009; Saravi *et al.*, 2012; Kasina *et al*., 2014; Goiato *et al*., 2015; Lima *et al*., 2016).

Denture Stomatitis is described as an inflammatory condition of the palatal mucosa that is delimited by the borders of the denture (Burket *et al*., 2008). Usually, it is asymptomatic, with just a few patients complaining about pain, itching or burning sensation (Wiens *et al*., 2018). The prevalence varies between 25 to 70%, according to different studies (Devlin *et al*., 2012; Salim *et al*., 2012; Lima *et al*., 2016) and depending on the population incurred (Devlin *et al.*, 2012). This pathology is frequent in elderly patients, especially those that follow a continuous use of the prosthesis, are less capable to perform the denture hygiene and immunocompromised (Radnai *et al*., 2009; Neville *et al*., 2011; Salim *et al*., 2012).

In spite the etiology is not completely clear, Denture Stomatitis is considered a clinical finding of Erythematous Candidiasis or Chronic Atrophic Candidiasis, as referred in the previous nomenclature (Neville *et al.*, 2011), a subgroup of Oral Candidiasis (Amin *et al*., 2009). *Candida albicans* is considered the major responsible of Oral Candidiasis, as well as of Denture Stomatitis (Bertolini *et al*., 2014), but there are some other Candida species that can be isolated from these lesions, namely *Candida tropicalis, Candida krusei, Candida glabrata*, among others (Burket *et al*., 2008; Amin *et al*., 2009; Boscato *et al*., 2009; Salim *et al*., 2012). The etiology is controversial since it is not certain if this condition represents an infection caused by *Candida albicans* or a host tissue reaction due to the diverse colonization on the denture surface (Neville *et al*., 2011). When a denture surface and the underlying mucosa are scrapped, in order to raise a separately culture in a sabouraud agar medium, the denture surface culture presents a higher fungal colonization than the one from the mucosa (McCourtie *et al.*, 1986; Neville *et al.*, 2011).

Treatment of Denture Stomatitis evolves topical or systemic antifungal drugs. Nevertheless, they are associated to relapses, since *Candida albicans* seems to penetrate the denture acrylic (Devlin *et al*., 2012). To counteract relapses of Denture Stomatitis, incorporation of antifungal drugs in the acrylic resins has been studied (Radnai *et al*., 2009; Salim *et al.*, 2012).

Chlorhexidine was also studied and is presenting currently the best results when microbiologic tests are concerned (Amin *et al*., 2009; Radnai *et al*., 2009; Ryalat *et al*., 2011). Chlorhexidine is an antimicrobial agent widely used for oral health maintenance and also for the treatment of oral infections (Rosa *et al.*, 2015). Also, appears to reduce the activity of

Candida albicans, being more effective than topical fluconazole, since it can damage the cytoplasmic membranes in yeasts (Lorian, 2005; Ryalat *et al*., 2011; Salim *et al.*, 2012; Salim *et al.*, 2013). The most common form of CHX application is through a 0.2% mouth rinse, allowing the reduction of microbial burden, related to biofilms (Imbert, 2016). However, its efficiency is influenced by not only its concentration but also its exposure time, which can be associated to a therapeutic failure caused by the turnover effect of saliva and the cleansing action of oral musculature (Imbert, 2016). CHX loading on resins has a slower and constant release of CHX into the oral cavity until twenty eight days (Amin *et al.*, 2009; Ryalat *et al.*, 2011; Salim *et al.*, 2012; Marcelino, 2015), being more effective than the mouth rinse.

It has to be taken into account that CHX loading can influence the physical properties of the acrylic resins (Sousa, 2014; Bertolini *et al.*, 2014; Martins, 2015). Also a long use of the denture in the oral cavity can implicate the total elution of the CHX, within a time interval of approximately three months (Gale and Darvell, 1999; Sousa, 2014). The biodegradation of the acrylic resin promoted by breathing, eating and drinking (Palmer *et al.*, 1992), as well as the saliva components should be mimetized to understand the behavior of the material overtime. Saliva is majorly composed by water, which will penetrate the resin and allow the release of non-cured monomer and the elution of CHX (Kawahara *et al.*, 2004; Neves, 2012). This elution will leave behind porosities that can affect the physical and mechanical properties such as shear bond strength and surface free energy, two properties that influence the performance of the dentures (Radnai *et al.*, 2009; Kasina *et al.*, 2014; Lima *et al.*, 2016).

The bonding procedure of the reline resin to the denture base resin must allow a high performance of the prostheses. It is suggested that direct reline resins are more susceptible to biodegradation compared to indirect reline resins; however, there are advantages that prevail, as time and logistics, which empathize their importance (Neves, 2012). The conditions inside the oral cavity may reduce the shear bond strength between the denture base resin and the relining material, much probably associated to the water sorption in the bonding interface (Polyzois, 1992). A weak bond will permit increase of bacteria, debonding of the reline resin from the denture base and staining (Takahashi *et al.*, 2001a; Takahashi *et al.*, 2001b; Lau *et al.*, 2013).

Concerning surface free energy, this property can be calculated according to the contact angle of a solid subjected to two different liquids (Ozden *et al.*, 1999; Bettencourt *et al.*, 2002; Costa, 2014; Barreiros, 2015). It is know that a higher surface free energy evolves a higher wettability, which is associated to an increase of adherence of *Candida albicans* (Minagi *et al.*, 1985; Prasad *et al*., 2012) but it is also important to retain that wettability is responsible for a good retention of the denture and absence of frictional problems and mucosal trauma (Ozden *et al.*, 1999; Jin *et al.*, 2009).

This investigation seeks to clarify the behavior of acrylic reline resins when loaded with CHX after one month inside the oral cavity and subjected to thermal fluctuations, since few evidence is available.

2. Objectives

The first main goal of this study was to evaluate the influence of loading three acrylic reline resins with different concentrations of CHX in terms of shear bond strength, after a thermal ageing process, according to the following hypothesis:

$H0_1$: Loading Kooliner with various concentrations of CHX doesn't affect the shear bond strength to the acrylic base resin.

$H1_1$: Loading Kooliner with various concentrations of CHX affects the shear bond strength to the acrylic base resin.

$H0_2$: Loading Ufi Gel Hard with various concentrations of CHX doesn't affect the shear bond strength to the acrylic base resin.

$H1_2$: Loading Ufi Gel Hard with various concentrations of CHX affects the shear bond strength to the acrylic base resin.

$H0_3$: Loading Probase Cold with various concentrations of CHX doesn't affect the shear bond strength to the acrylic base resin.

$H1_3$: Loading Probase Cold with various concentrations of CHX affects the shear bond strength to the acrylic base resin.

The other purpose of this investigation was to evaluate the influence of loading the same three acrylic reline resins with different concentrations of CHX in terms of surface free energy after a thermal ageing process, according to the following hypothesis:

$H0_4$: Loading Kooliner with various concentrations of CHX doesn't affect the surface free energy.

$H1_4$: Loading Kooliner with various concentrations of CHX affects the surface free energy.

$H0_5$: Loading Ufi Gel Hard with various concentrations of CHX doesn't affect the surface free energy.

$H1_5$: Loading Ufi Gel Hard with various concentrations of CHX affects the surface free energy.

$H0_6$: Loading Probase Cold with various concentrations of CHX doesn't affect the surface free energy.

$H1_6$: Loading Probase Cold with various concentrations of CHX affects the surface free energy.

3. Materials and Methods

3.1. Materials

Three autopolymerizing acrylic reline resins based on a liquid-powder mixture, where selected for this study: two of them were direct reline resins, Kooliner (GC America Inc., Alsip, Illinois, USA) and Ufi Gel Hard (Voco GmbH, Cuxhaven, Germany) and the last one associated to an indirect polymerization process, Probase Cold (Ivoclar Vivadent AG, Liechtenstein). The characteristics of each of these materials are presented on Table 3.1.

Table 3.1 – Materials selected for the study.

Product	Composition		Powder/ Liquid Ratio	Polymerization Condition	Curing Cycle	Manufacturer	Batch Number
	Powder	Liquid					
Kooliner (K)	PEMA	IBMA	1.4 / 1	Autopolymerization	10 min 37ºC	GC America Inc., Alsip, Illinois, USA	1703062 (P) 1704191 (L)
Ufi Gel Hard (UF)	PEMA	1,6-HDMA	1.77 /1	Autopolymerization	7 min 37ºC	Voco GmbH, Cuxhaven, Germany	1735175 (P) 1739457 (L)
Probase Cold (PC)	PMMA	MMA	1.5 / 1	Autopolymerization	15 min 40ºC 2-4 bar	Ivoclar Vivadent AG, Liechtenstein	WT0487 (P) W85050 (L)

P = Powder, L = Liquid; PMMA = Polymethylmethacrylate: MMA = Methylmethacrylate, PEMA = Polyethylmethacrylate, IBMA = Isobuthylmethacrylate, HDMA = Hexanedioldimethacrylate, min = minutes.

The CHX selected for this study was Chlorhexidine Diacetate Monohydrate (Panreac AppliChem, Darmstadt, Germany). Concentrations of CHX were chosen according to previous studies (Sousa, 2014; Martins, 2015).

The proportions were measured according to the acrylic reline resin weight (w/w). For the experimental groups, a mortar and a pestle were required to homogenize the powder mixture of acrylic reline resin and CHX. By this time, the mixture was blend with the correspondent amount of liquid. Concerning the direct acrylic reline resins, polymerization was obtained at oral cavity temperature, namely 37ºC, with the recommended time stated by the manufacturer. To achieve the polymerization of Probase Cold groups, the relined specimens were placed inside an Ivomat pressure device (IvoclarVivadent, Liechtenstein) during 15 min at a temperature of 40ºC and 2-4 bar.

3.2. Shear bond strength

Preparation of denture base specimens

First, one hundred and twenty specimens of heat-polymerizing denture base acrylic resin Probase Hot (Ivoclar Vivadent AG, Liechtenstein) were prepared using a modified flasking technique. A silicon mold was used to elaborate wax specimens with a rectangular shape (12 × 10 × 6 mm). All of them were flasked and placed, afterwards, above the investment with gypsum type II**.** Later on, the primary stratum of gypsum was coated with vaseline, placing another compound of gypsum type II and III mixture on the superior half, covering the specimens. Prior setting the second stratum, the top of the flask was positioned in order to allow the flowing of excess of gypsum throw the holes. After the complete set of the gypsum was achieved, the flask was placed under boiling water between 4 to 6 minutes. After, it was removed from the boiling water and then opened to clear the wax. Applying a separating fluid on the impressed gypsum (Ivoclar Vivadent AG, Liechtenstein), the heat-polymerizing resin, Probase Hot, composed by prepolymerized polymethylmethacrylate powder and methylmethacrylate liquid, was manipulated and packed into the flask with a powder/liquid ratio of 22.5/10 g/mL, undergoing polymerization through a hydraulic system which granted the conditions according to the manufacturer's instructions (heat up to 100ºC and let boil for 45 min). Ultimately, and before removing the specimens, the flasks were withdrawn from the water and let cooled in order to stabilize at room temperature.

To be possible to recreate the conditions inside the oral cavity, the specimens were submitted to a thermocycling machine (Refri 200-E, Aralab, Cascais, Portugal), that exposed them to a thermal ageing procedure, compound by 2500 cycles with thermal fluctuations, namely 5ºC and 55ºC (20 seconds each bath), accomplishing an interval of 5 seconds between each bath.

Relining procedure

Preceding relining of the specimens, a reduction to a 3mm thickness was done in a rotational grinding and polishing machine (DAP- U, Struers, Denmark) with a 600-grit

silicon carbide paper (Carbimet Paper Discs, Buehler Ltd., Lake Bluff, IL). The thickness was confirmed using a digital micrometer (Mitutoyo Digimatic, MFG.Co, Ltd. Tokyo, Japan) with a precision of ± 0.01 mm.

A perforate adhesive tape (Glossy White Film EA, Xerox) was customized and placed on the top of each specimen to define a bonding area of 3mm (Figure 3.1).

One hundred and twenty denture base specimens were randomly divided in groups (*n*=10), resulting in 4 groups for relining with Kooliner, 5 groups with Ufi Gel Hard and 3 groups of Probase Cold (according to the proportions of CHX).

The concentrations of CHX selected for loading in each material are described on Table 3.2.

Table 3.2 – Materials and correspondent CHX percentages.

% CHX / Resin	0%	2.5%	5%	7.5%	10%
Kooliner	✓	✓	✓	✓	
Ufi Gel Hard	✓	✓	✓	✓	✓
Probase Cold	✓	✓	✓		

Prior relining the Kooliner and Probase Cold specimens, and according to the manufacturer's recommendations, correspondent monomer of these reline resins was soaked on the bonding area. Denture base specimens allocated to the Ufi Gel Hard groups were subjected to a specific conditioner and then dried in the air for about 30 seconds, as recommend by the manufacturer. A rectangular shape silicon mold with a circular opening (5mm internal diameter × 3mm height) was placed atop the perforated adhesive tape, to establish the relining area.

The relining procedure was executed as described above, according to the manufacturer's instructions (Figure 3.2).

Ending the curing cycle, the relined samples were displaced from the mold and single plan lap and then submitted to a thermocycling procedure (Refri 200-E, Aralab, Cascais, Portugal) of 1000 cycles with thermal fluctuations, namely 5ºC and 55ºC (20 seconds each bath), accomplishing an interval of 5 seconds between each bath.

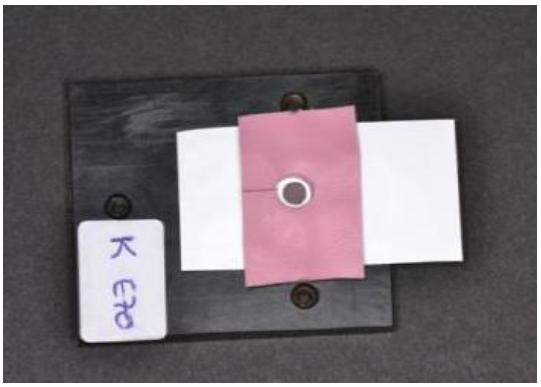

Figure 3.1 – Shear bond strength device with the adhesive tape and the silicon mold.

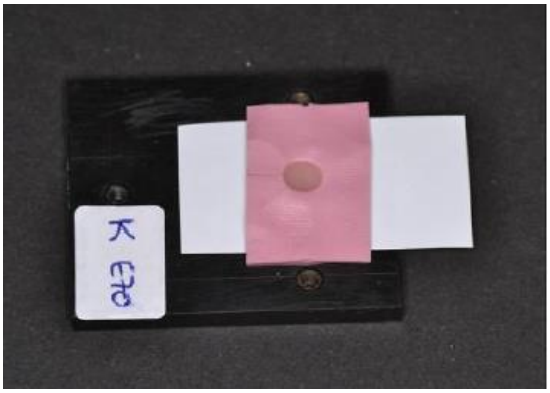

Figure 3.2 – Shear bond strength device relined with Kooliner.

Shear bond strength test

Specimens were individually attached to a single plan lap with gypsum type IV (Figure 3.3), with another single plan lap atop of them and then installed in a universal testing machine model 4502 (Instron Ltd., Bucks, HP 12 3SY, England).

Shear bond strength was assessed with specific features, 1kN load cell and crosshead speed of 1mm/min until the separation of the denture base resin and the reline resin (Figure 3.4). The tests were performed through atmospheric conditions at room temperature.

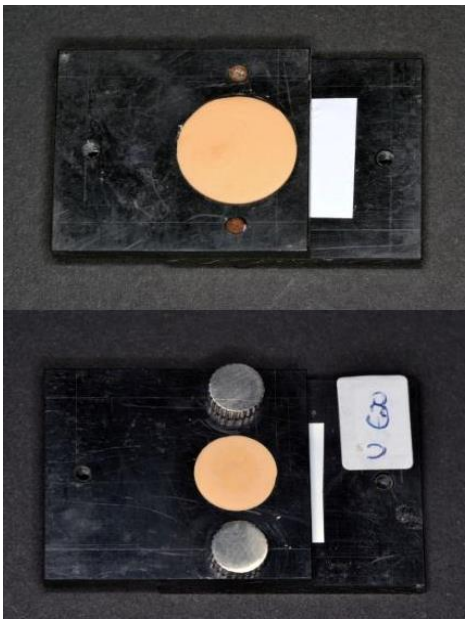

Figure 3.3 – Shear bond strength device with gypsum.

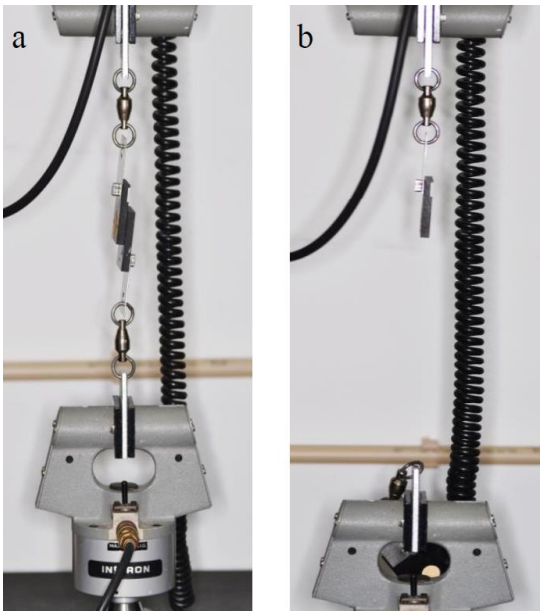

Figure 3.4 – An example of specimen submitted to shear bond strength test: a) Before test; b) After test.

Failure mode assessment

After testing shear bond strength, a stereomicroscope (EMZ-8TR, Meiji Techno Co, Saitama, Japan) was used to evaluate the failure mode on the two detached surfaces of each specimen (Figure 3.5). The failure mode was classified as adhesive, cohesive or mixed by two independent observers. To be classified as adhesive, the denture base resin debonded surface should not have trace of reline resin. To be considered mixed, vestiges of reline resin should be present. Finally, to be allocated as cohesive, the debonded denture base surface should be fulfilled with reline resin.

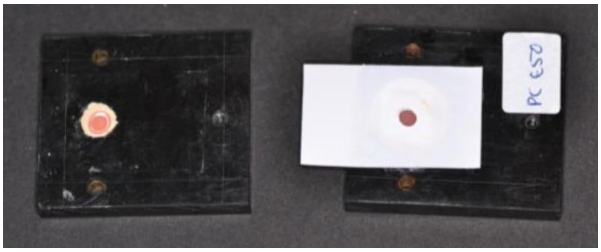

Figure 3.5 – Shear bond strength device after being submitted to shear bond strength test.

3.3. Surface free energy

Preparation of specimens

Three groups of seven specimens in each material were produced, accordingly to specific concentrations of CHX (w/w) loaded, as demonstrated in Table 3.3, and precluding a total of sixty three specimens were produced.

Table 3.3 – Materials and correspondent CHX percentages.

% CHX / Resin	0%	2.5%	5%	7.5%	10%
Kooliner	✓	✓	✓		
Ufi Gel Hard	✓		✓	✓	
Probase Cold	✓	✓	✓		

To prepare the specimens, metallic rectangular shapes (125 × 25 × 1 mm) were used. According to the manufacturer's instructions and following the polymerization recommendations for each material mentioned above, the acrylic resins loaded with CHX were placed inside the metallic molds (Figure 3.6 and 3.7). In order to remove the excess of material, the molds were covered with a lid, allowing the excess to spread. After polymerization, all the samples were removed from the molds and cut to the dimensions of approximately 25mm width, 16mm height and 1mm thickness. Specimens were cut with a turbine cylindrical drill.

At this point, the specimens were submitted to a thermocycling procedure, compound by a 1000 cycles with thermal fluctuations, namely 5ºC and 55ºC (20 seconds each bath), accomplishing an interval of 5 seconds between each bath.

Then, the edges of all specimens were polished with a 600-grit silicon carbide paper (Carbimet Paper Discs, Buehler Ltd., Lake Bluff, IL) to eliminate irregularities.

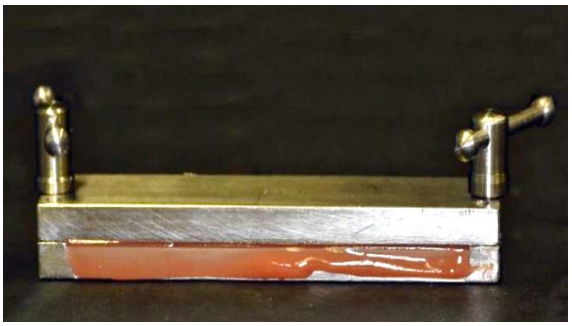

Figure 3.6 - Compression of one resins dough in the metal mold.

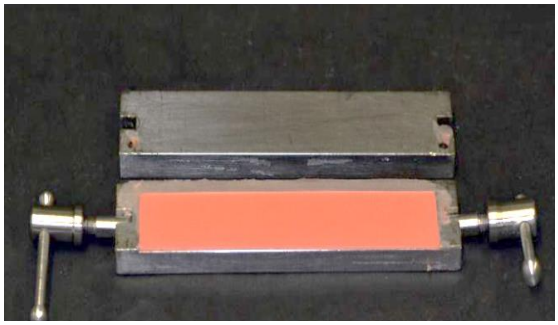

Figure 3.7 – Metal mold opened after cure of the acrylic reline resin.

Surface free energy assessment

To access the surface free energy of the specimens, a Tensiometer K12 (Kruss, Hamburg, Germany) connected to a computer was required. The results of each group were obtained applying the Wilhelmy plate technique (Bettencourt, *et al*., 2004), that allowed the measure of the contact angles of distilled water and 1,2-propanediol on 5 specimens of each group at room temperature.

With a digital micrometer (Mitutoyo Digimatic, MFG.Co, Ltd. Tokyo, Japan) (with a precision of ± 0.01mm) height, width and thickness of each specimen were measured and introduced in a software. Firstly, the specimen was left suspended on the balance (sensivity = 10^{-4} g) of the equipment, following the immersion of 4mm in the liquid (water or 1,2-propanediol) at a speed of 20µm s^{-1}.

In order to estimate the surface free energy (γ) of the specimens, as well as its dispersive (γ^d) and polar (γ^p) components, based on the harmonic mean method of Wu (1971), advancing contact angles were used. The KRUSS-software program - contact angle measuring system K121 (version 2.049) – was required for the equations of surface free energy estimation.

The 1,2-propanediol chosen for this study had a total surface free energy (γ) of 38 mN/m, with a dispersive component (γ^d) of 28.6 mN/m and a polar component (γ^p) of 9.4

mN/m. The density was 1.04 kg/m^3 and the respective molar mass was 76.09 g/mol (1-2 Propanediol R.822324-1L; Merck, Germany).

The water used in surface energy assessment (3.3) was of Milli-RX quality (Merck Millipore, Germany).

3.4. Statistical analysis

All data were statistically analyzed with SPSS Statistics 20 (SPSS Inc., Chicago, IL, USA). The results were submitted to the nonparametric tests according to the Kruskal-Wallis method, followed by multiple comparisons using Man-Whitney test with Bonferroni correction, since data did not follow a normal distribution in the Shapiro-Wilk normality tests. It was considered a 5% level of significance in all statistical tests (α=0.05).

4. Results

4.1. Shear bond strength

Results of the shear bond strength are summarized in Table 4.1, as well as the mean (M), standard deviation (SD), median (m) and interquartile range (IR) values per group of CHX loaded.

Table 4.1 – Shear bond strength data by reline resin (*n*=10).

Material	% CHX loaded	Shear Bond Strength (MPa)		
		M(SD)	m	IR
Kooliner	Control - 0%	5.4(3.25)	4.92	2.73
	2.5%	8.3(4.58)	7.79	5.43
	5%	4.7(1.73)	4.36	2.79
	7.5%	5.1(2.14)	4.98	3.17
Ufi Gel Hard	Control - 0%	9.8(5.11)	9.11	7.80
	2.5%	7.8(4.13)	6.26	4.50
	5%	12.3(4.76)	11.25	4.47
	7.5%	9.4(2.40)	9.70	2.58
	10%	16.6(7.78)	14.13	13.62
Probase Cold	Control - 0%	25.9(8.48)	26.56	16.25
	2.5%	17.3(5.31)	16.95	5.73
	5%	16.2(5.02)	15.57	10.41

M=Mean; SD=Standard deviation; m=Median; IR=Interquartile range.

Concerning Kooliner specimens (Figure 4.1), no statistical differences were observed between the groups ($p>0.05$).

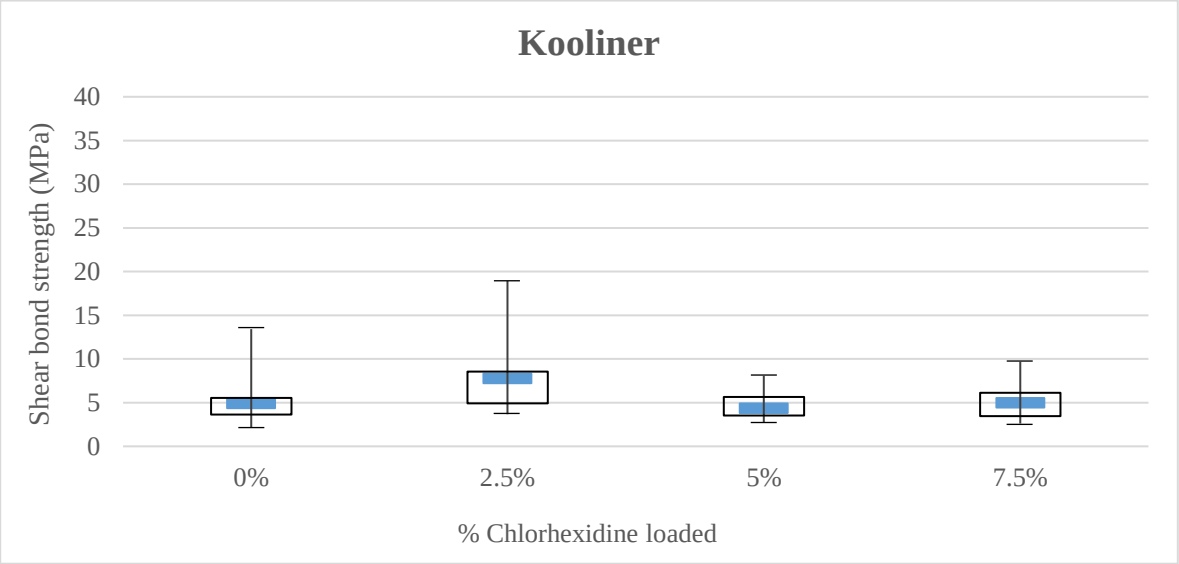

Figure 4.1 – Median and Interquartile Range of Kooliner groups for shear bond strength.

Regarding Ufi Gel Hard specimens (Figure 4.2), there were no differences between experimental groups and the control. Only 10% CHX group showed higher values than 2.5% group (p=0.009).

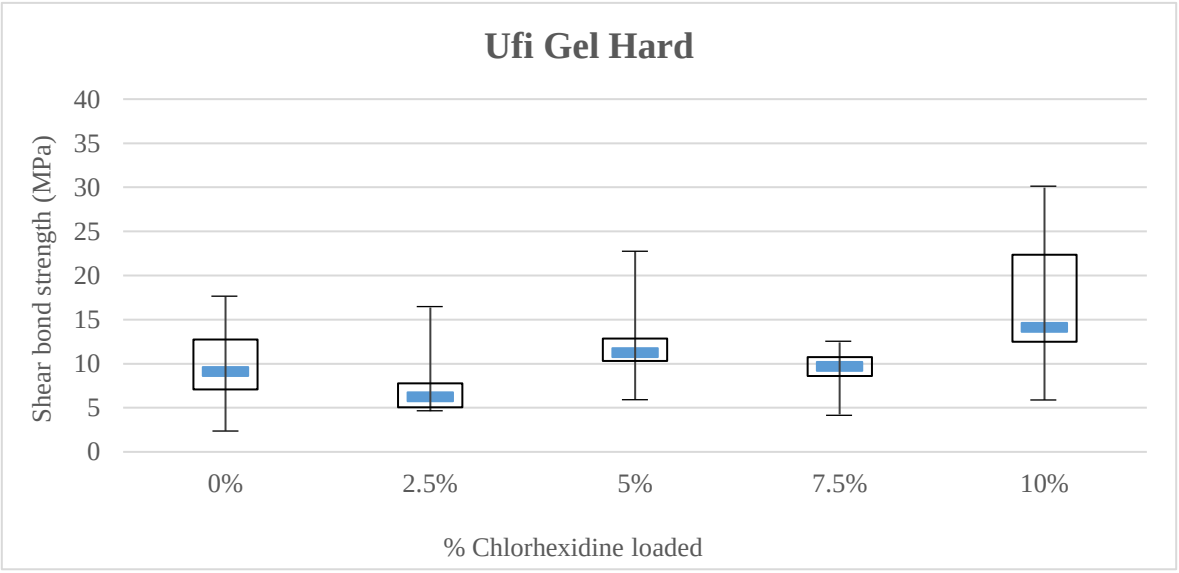

Figure 4.2 – Median and Interquartile Range of Ufi Gel Hard for shear bond strength.

According to the statistical data obtained, in Probase Cold groups (Figure 4.3), statistical differences were found (p=0.033), with 5% CHX group suggesting lower values than the control group. No other differences were found.

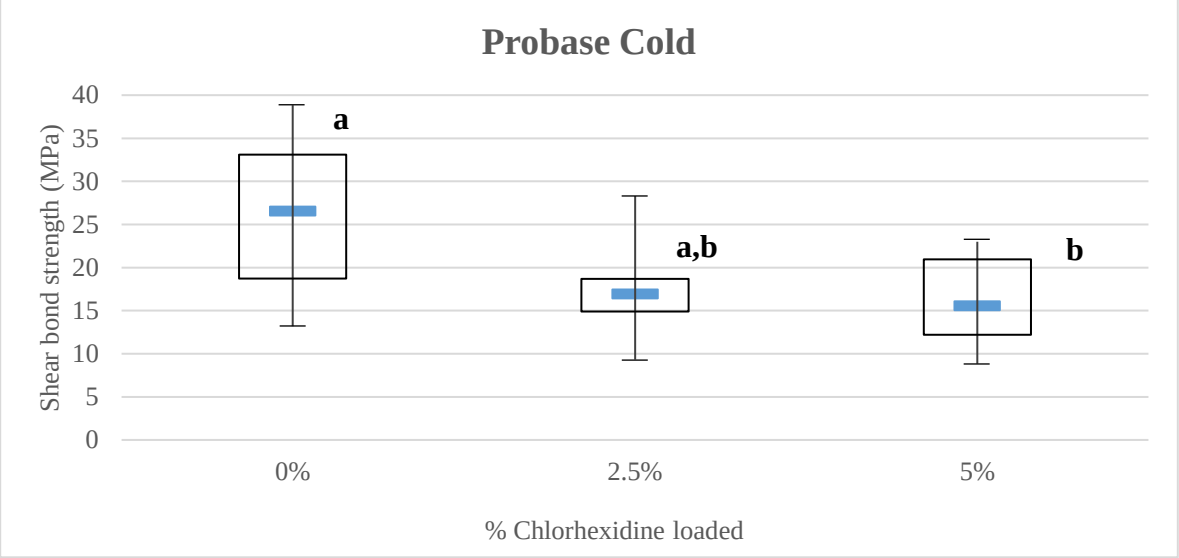

Figure 4.3 – Median and Interquartile Range of Probase Cold for shear bond strength. Groups assigned with the same letter (a/b) show no statistical significant differences among them.

All specimens were observed in a stereomicroscope to assess the type of bonding failure. The percentages of failure inside each group of the three acrylic reline resins are stated in Table 4.2. Kooliner specimens presented 100% adhesive failure, while Ufi Gel Hard had an increase from the control group to the loaded CHX groups of adhesive failure to a mixed failure. Finally, Probase Cold showed a decrease of cohesive failure from the control group to the loaded CHX groups.

Table 4.2 – Percentage of Failure according to the acrylic reline resin and proportion of CHX loaded.

Material	% CHX loaded	% of Failure		
		Adhesive	Mixed	Cohesive
Kooliner	0%	100%	-	-
	2.5%	100%	-	-
	5%	100%	-	-
	7.5%	100%	-	-
Ufi Gel Hard	0%	100%	-	-
	2.5%	100%	-	-
	5%	70%	30%	-
	7.5%	60%	40%	-
	10%	60%	40%	-
Probase Cold	0%	30%	30%	40%
	2.5%	50%	40%	10%
	5%	60%	40%	-

- : 0%.

4.2. Surface free energy

Descriptive analysis of the data was performed for each one of the three materials, including mean, standard deviation, median and interquartile range values for contact angle (Appendix 1, Tables 1, 2,3 and 4).

The values of the total surface free energy and their respective components, the dispersive (γ^d) and polar (γ^p), are summarized in Table 4.3, as well as the mean and standard deviations of the groups by reline resin.

Table 4.3 - Mean and standard deviation (SD) values for total surface free energy as well as the dispersive and polar components by reline resin.

Material	% CHX loaded	Surface Free Energy (γ) (mN/m)(SD)		
		γ Total	γ Dispersive	γ Polar
Kooliner	Control - 0%	30.0(5.68)	9.8(4.79)	20.2(10.17)
	2.5%	26.5(1.47)	9.8(3.87)	16.7(4.87)
	5%	26.9(2.88)	10.7(0.62)	16.2(2.76)
Ufi Gel Hard	Control - 0%	28.1(5.24)	10.3(6.06)	17.8(8.21)
	5%	31.4(6.91)	9.9(5.46)	21.5(11.05)
	7.5%	28.9(2.63)	11.0(3.62)	18.0(5.21)
Probase Cold	Control - 0%	26.8(1.13)	8.5(4.20)	18.4(4.52)
	2.5%	27.8(1.73)	8.2(3.41)	19.7(3.80)
	5%	28.0(3.61)	11.0(1.58)	17.1(5.08)

γTotal=Total surface free energy; γDispersive=Dispersive surface free energy; γPolar=Polar surface free energy;

In respect Kooliner specimens (Figures 4.4; Appendix 7, Figures 16 and 17), there were no significant differences in total surface free energy and either in the dispersive (γ^d) and polar components (γ^p).

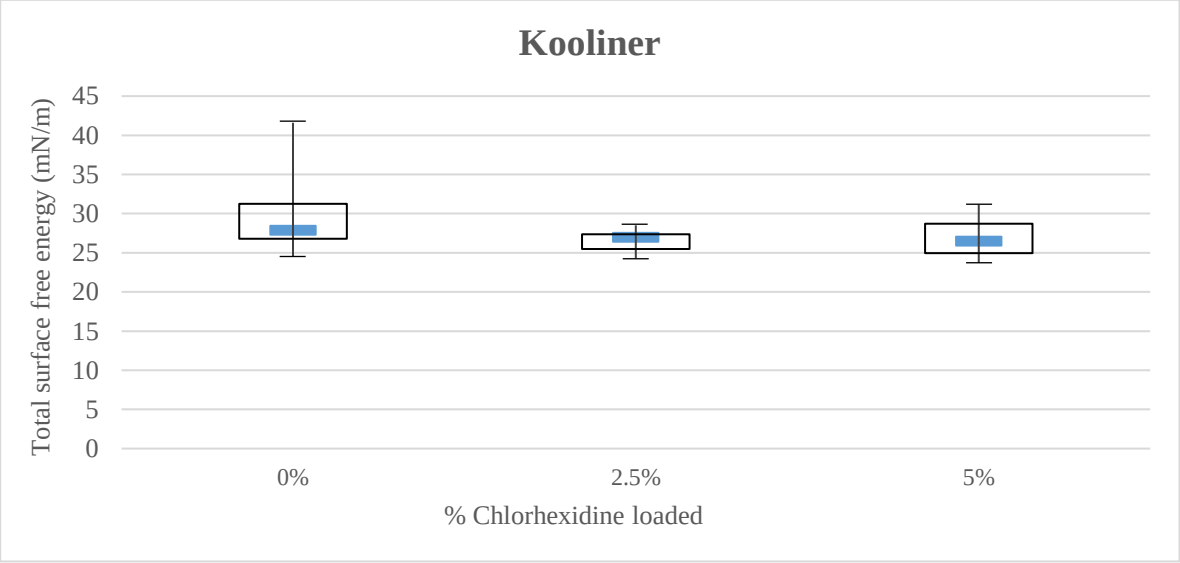

Figure 4.4 – Median and Interquartile Range of Kooliner for total surface free energy.

Considering the Ufi Gel Hard specimens (Figure 4.5; Appendix 7, Figures 18 and 19), significant differences have not occurred either in the total surface free energy or in its correspondent components, dispersive (γ^d) and polar (γ^p) (p>0.05).

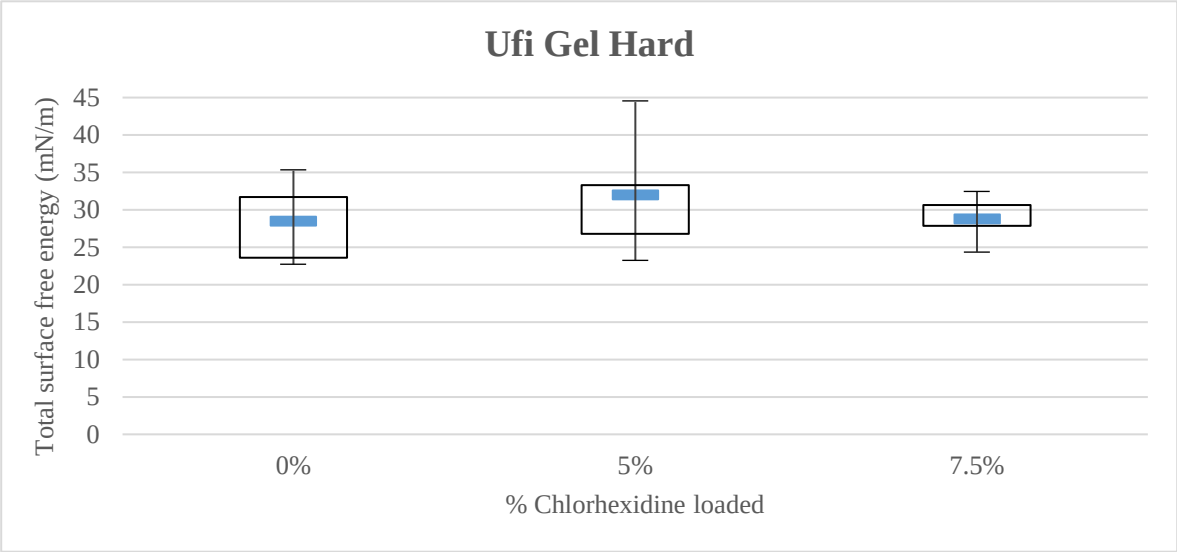

Figure 4.5 – Median and Interquartile Range of Ufi Gel Hard for total surface free energy.

Regarding Probase Cold specimens (Figure 4.6; Appendix 7, Figures 20 and 21), as well as in the previous groups, there were no statistical differences in total surface free energy and in the dispersive (γ^d) and polar (γ^p) components (p>0.05).

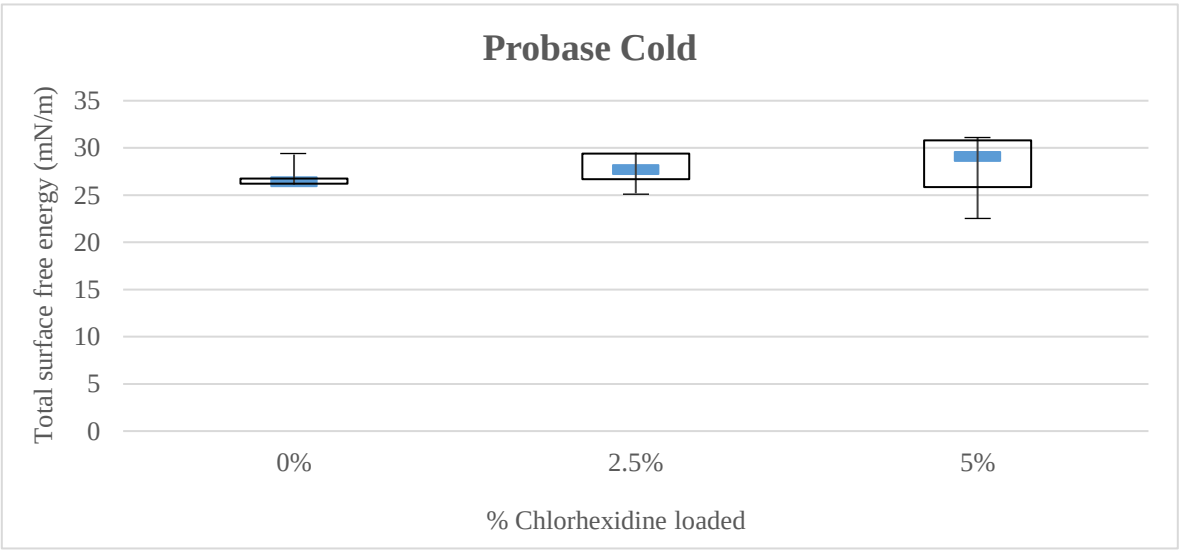

Figure 4.6 – Median and Interquartile Range of Probase Cold for total surface free energy.

5. Discussion

Loading CHX in resins have shown to be efficient in reducing the proliferation of *Candida albicans* (Amin *et al.*, 2009; Radnai *et al.*, 2009; Ryalat *et al.*, 2011) and, therefore, should have the potential of prevent or treat denture stomatitis. Though it is understood as a promising treatment solution, it has to be taken into account that drug loading can weaken the physical and mechanical properties of the biomaterials (Sousa, 2014; Barreiros, 2015; Martins, 2015).

On the present study, three acrylic resins loaded with chlorhexidine were studied in order to check if this drug loading could negatively affect the properties of the resins. These materials were chosen for their differences in chemical composition and structural arrangement, two factors that determine the physical properties of a biomaterial.

Two of them are based on poly(ethyl methacrylate) since represent direct reline resins (oral polymerization) – Kooliner and Ufi Gel Hard- and one is based on poly(methyl methacrylate), representing an indirect reline resin (laboratory polymerization)- Probase Cold. All the materials, are based in distinct monomers: isobutylmethacrylate (Kooliner), 1,6-hexanodioldimethacrylate (Ufi Gel Hard) and methylmethacrylate (Probase Cold) (Arima *et al.*, 1995; Arima *et al.*, 1996).

Also, cross linking materials are based on bimonomers, such as 1,6-hexanodioldimethacrylate from Ufi Gel Hard, that has a complex structural arrangement and forms complex net with large molecules (Anusavice *et al.*, 2014).

Since these resins have different physical structure and chemical composition, CHX molecules incorporated on the net can create distinct links in the polymer molecules that can change their properties in distinct magnitudes. Also, they can increase the distance between molecules, resulting in an expected weaker polymer net.

The physical and mechanical properties of the acrylic reline resins under study were already investigated (Sousa, 2014; Barreiros, 2015; Martins, 2015). Nevertheless, these studies focused on measuring the properties of chlorhexidine loaded resins without any influence of the environment.

An important issue regarding the clinical application of the acrylic reline resins is their biodegradation. It can be defined as the changes on their chemical, physical and mechanical properties due to the oral environment conditions (Neves, 2012). One of the causes of biodegradation is the thermal changes promoted by breathing, eating and drinking (Palmer *et al.*, 1992). In *in vitro* studies, ageing processes should be applied to understand the biomaterial behavior overtime.

To perform the oral biodegradation process, in the present study, specimens were submitted to a thermal ageing process through a thermocycling procedure that consisted on 1000 cycles of alternative immersion of the specimen on 5ºC and 55ºC baths. This procedure simulates the oral thermal changes for one month, since it has been shown that once CHX is incorporated into polymethylmethacrylate, it retains its therapeutic dose for up to 28 days (Amin *et al.*, 2009; Salim *et al.*, 2012)

This process also can mimic the aqueous environment of the oral cavity. Saliva is majorly composed by water, which will penetrate the resin and allow the release of non-cured monomer and the elution of CHX (Kawahara *et al.*, 2004; Neves, 2012). This elution will leave behind porosities that can affect properties such as shear bond strength and surface free energy, two properties that influence the performance of the dentures (Radnai *et al.*, 2009; Kasina *et al.*, 2014; Lima *et al.*, 2016).

Shear bond strength is mentioned to be related with the chemical properties of the bonded resins (Lau *et al.*, 2013). A weak bond will implicate proliferation of bacteria, the separation of the reline resin from the denture base and staining (Takahashi *et al.*, 2011a,b; Lau *et al.*, 2013).

In the present shear bond study, concentrations of CHX loaded on specimens were selected according to previous studies that showed not to negatively influence the mechanical and physical properties of each material (Sousa, 2014; Barreiros 2015; Martins, 2015). The concentrations of 10%, 7.5%, 5%, 2.5% and 1% of CHX were studied by these authors.

Sousa (2014) referred that loading 10% CHX could negatively influence microhardness and flexural strength of Kooliner and Probase Cold, but not Ufi Gel Hard, leading to the choice of including in the present study the 10% CHX group only in Ufi Gel

Hard. Martins (2015) found that a 7.5% concentration would decrease flexural resistance on Probase Cold, so it was settled in the present study to include groups of Probase Cold only till 5% CHX. Also, 1% CHX groups were eliminated from all the materials since it was proved not to have anti-microbiological activity against *Candida Albicans* (Costa *et al*., 2017).

The first objective of this study was to evaluate whether the loading of CHX affects or not the shear bond strength.

In the present study, there were no statistical differences found between Kooliner experimental groups and the control. These results are in conformity with those found by some other authors (Alcântara *et al*., 2012; Barreiros, 2015). Barreiros (2015) studied the shear bond strength of Kooliner specimens immediately after the polymerization process, having the same results as the present study, where the specimens were tested after thermal ageing. Considering the fact that Kooliner, an autopolymerizing reline resin, is associated to higher levels of non-cured monomer (Jorge *et al.,* 2004), we can assume that the loading of CHX plays the same role of the non-cured monomer. As the percentages of CHX are very low, statistically they seem not to be representative for shear bond strength with or without thermal ageing procedure. The type of failure in Kooliner specimens was 100% adhesive, which means that this type of bonding may reveal to be weak and require a more often relining of the denture base. This can be related to the type of monomer that composes Kooliner, isobutylmethacrylate, a high molecular monomer, as far as it is stated that high molecular monomers have more difficulty to penetrate the base resin (Ahmad *et al.*, 2009; Giampaolo *et al.,* 2011; Costa, 2014). At this point, the null hypothesis can be accepted, since there were no differences between shear bond strength of experimental groups compared to the control.

Still, Ufi Gel Hard groups have not presented significant differences among them, only 10% CHX group presenting higher values of shear bond strength than the 2.5% CHX group. This is an interesting finding as Sousa (2014) and Martins (2015) also found that CHX loading of Ufi Gel Hard reline resin would positively increase the properties of the material, namely the microhardness. It is also stated that thermal ageing can slightly increase the tensile strength of a product from the same range of Ufi Gel Hard, namely, Ufi Gel C (Kulak-Ozkan

et al., 2003). Also the type of failure varies between adhesive and mixed, besides the fact that Ufi Gel Hard is an autopolymerizing resin and has a high molecular monomer, 1,6-hexanedioldimethacrylate. This means that, besides preventing and treating denture stomatitis, the loading of Ufi Gel Hard with CHX can benefit its physical properties. More studies with a higher number of specimens are needed to confirm the conclusions of the present study. At this point, the second null hypothesis can't be rejected, as the loading of CHX seems to not affect the shear bond strength between the acrylic reline resin Ufi Gel Hard and the acrylic base resin.

Concerning Probase Cold specimens, 5% CHX group revealed to have lower shear bond strength values when compared to the control group. These results are correlated with the study of Barreiros (2015), which means that even with thermal ageing (that is expected to promote an additional polymerization of the resin), the loading of CHX at a 5% concentration negatively affects the shear bond strength to denture base resin. The type of failure also varies in Probase Cold specimens, with the percentage of cohesive and mixed failure reducing from the control to the CHX loaded groups. Regarding the fact that this reline resin evolves a low molecular monomer, methylmethacrylate, the percentage of cohesive or mixed failure should be higher. With this knowledge, the third null hypothesis can also be rejected, to be specific, loading Probase Cold with various concentrations of CHX seems to affect the shear bond strength with the acrylic base resin.

The other purpose of this investigation was to assess whether the loading of CHX would interfere or not with the surface free energy of the reline resins evolved in this study, after thermal ageing procedure.

Surface free energy can be assessed by the interaction of more than one liquid against the surface of a solid that stablish a certain contact angle, acknowledge with the polarity and surface tension of both. It is known that surface free energy can influence the colonization process *of Candida albicans* (Minagi *et al.*, 1985; Prasad *et al.*, 2012; Silva *et al.*, 2013). Higher values of surface free energy implicate an increase of adherence of this fungus.

In the present study, the results showed that there were no statistical differences in total surface free energy, dispersive and polar components between different groups in all reline resins ($p>0.05$). The fact that this study is protocolized with a thermal ageing process,

where the specimens underwent through a wet environment, the results suggest a complete elution of CHX from the reline resin, remaining only resin. This goes along with the results found by Barreiros (2015), as far as in the referred study the sample did not face any water solution, with the reline resin preserving the CHX, which led to higher surface free energy values in Ufi Gel Hard and Probase Cold groups. On the opposite side, Barreiros found differences inside Kooliner, between surface free energy and its dispersive and polar groups, with an absence of equilibrium among the components, justifying them with a probably irregular distribution on the particles within the material.

With this knowledge, all the fourth, fifth and sixth null hypothesis concerning surface free energy couldn't be rejected and, to be specific, the loading of CHX doesn't affect the surface free energy of the acrylic reline resin Kooliner, Ufi Gel Hard and Probase Cold.

Still respecting to Kooliner, it was observed an alteration on the configuration of all specimens after thermal ageing procedure (Appendix 2, Figure 14), namely a plastic deformation. It is known that water sorption can affect the mechanical properties of the resins and it is stated that it has a plasticizing effect on them (Koran III, 2002; Mese *et al*., 2008; Neves, 2012). This finding lead us to consider that this resin, when subject to oral cavity and ageing, besides the progressive bone resorption, will misfit quicker than the other two resins. It also has to be considered that autopolymerizing resins are associated to less cured monomer when compared with heat polymerizing resins, which means that there will be a loss of material to the evolving medium that can vary according to the chemical composition of different materials (Jorge *et al.,* 2004; Vergani *et al.,* 2005). No valuable evidence related to Kooliner was found to explain the plastic deformation specifically in this material, which means that more studies of its mechanical properties should be performed, including *in vivo tests*, which means to evaluate denture base resins that wherein need to be relined and then subjected to conditions inside the oral cavity. This kind of study could bring up a more realistic conclusion.

6. Conclusions

Considering the results obtained and the limitations of the study, the main conclusions are:

- Loading Kooliner with various concentrations of CHX doesn't seem to affect the shear bond strength to the acrylic base resin.

- Loading Ufi Gel Hard with various concentrations of CHX don't seem to affect the shear bond strength to the acrylic base resin, with only 10% CHX group presenting higher values than the 2.5% CHX group.

- Loading Probase Cold with various concentrations of CHX is suggested to affect the shear bond strength to the acrylic base resin, with 5% CHX group presenting lower values than the control.

- Loading Kooliner with various concentrations of CHX doesn't seem to affect the surface free energy.

- Loading Ufi Gel Hard with various concentrations of CHX do not demonstrate to influence the surface free energy.

- Loading Probase Cold with various concentrations of CHX do not shows to affect the surface free energy.

7. References

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Appendices

Appendix 1 – Tables

Table 1 - Contact angles by reline resin.

Material	% CHX loaded	Contact Angle (°)					
		Water			1,2 - Propanediol		
		M(SD)	Min	Max	M(SD)	Min	Max
Kooliner	Control - 0%	83.3(5.10)	77.6	90,7	66.9(15.96)	54.1	101.5
	2.5%	87.4(1.95)	84.5	90.4	67.9(11.96)	55.1	91.3
	5%	85.7(5.01)	78.7	91.7	63.6(3.49)	57.0	66.7
Ufi Gel Hard	Control - 0%	86.1(8.29)	74.6	93.1	67.4(12.59)	44.8	88.2
	5%	81.6(7.43)	75.1	92.1	66.2(18.11)	50.8	100.7
	7.5%	83.2(3.87)	79.0	90.6	62.3(11.00)	48.9	82.9
Probase Cold	Control - 0%	86.9(2.56)	82.4	90.6	71.7(13.05)	56.1	93.7
	2.5%	85.0(2.76)	81.2	88.4	71.3(11.55)	58.3	87.2
	5%	84.4(6.97)	78.6	95.5	62.9(2.04)	60.7	66.4

M=Mean; SD=Standard deviation; Min=Minimum; Max=Maximum.

Table 2 – Surface free energy data of Kooliner.

Material	% CHX loaded		Surface Free Energy (γ) (mN/m)		
			γ Total	γ Dispersive	γ Polar
Kooliner	Control - 0%	M±SD	30.0(5.68)	9.8(4.79)	20.2(10.17)
		m	27.90	11.10	19.60
		IR	5.00	4.10	9.00
	2.5%	M±SD	26.5(1.47)	9.8(3.87)	16.7(4.87)
		m	27.00	10.80	13.90
		IR	2.40	5.30	7.00
	5%	M±SD	26.9(2.88)	10.7(0.62)	16.2(2.76)
		m	26.50	10.60	15.90
		IR	6.40	0.70	5.90

M=Mean; SD=Standard deviation; m=median; IR=Interquartile range.

Table 3 – Surface free energy data of Ufi Gel Hard.

Material	% CHX loaded		Surface Free Energy (γ) (mN/m)		
			γ Total	γ Dispersive	γ Polar
Ufi Gel Hard	Control - 0%	M±SD	28.1(5.24)	10.3(6.06)	17.8(8.21)
		m	28.50	7.80	16.30
		IR	11.50	6.10	15.10
	5%	M±SD	31.4(6.91)	9.9(5.46)	21.5 (11.05)
		m	32.00	10.80	20.60
		IR	6.70	7.20	8.60
	7.5%	M±SD	28.9(2.63)	11.0(3.62)	18.0(5.21)
		m	28.80	12.00	15.10
		IR	3.60	4.20	6.00

M=Mean; SD=Standard deviation; m=median; IR=Interquartile range.

Table 4 – Surface free energy data of Probase Cold.

Material	% CHX loaded		Surface Free Energy (γ) (mN/m)		
			γ Total	γ Dispersive	γ Polar
Probase Cold	Control - 0%	M(SD)	26.8(1.13)	8.5(4.20)	18.4(4.52)
		m	26.40	6.90	19.70
		IR	0.80	6.10	7.70
	2.5%	M(SD)	27.8(1.73)	8.2(3.41)	19.7(3.80)
		m	27.70	9.10	18.20
		IR	3.40	7.40	5.80
	5%	M(SD)	28.0(3.61)	11.0(1.58)	17.1(5.10)
		m	29.10	10.40	20.00
		IR	7.70	3.10	10.00

M=Mean; SD=Standard deviation; m=median; IR=Interquartile range.

Table 5 – Measures of Kooliner specimens for surface free energy study.

% CHX loaded	Specimen Number	Measures (mm)		
		Width	Height	Thickness
Control 0%	1	24.79	16.38	1.10
	2	24.23	16.34	1.05
	3	24.27	16.81	1.09
	4	24.03	16.88	1.07
	5	24.06	18.23	1.00
	6	23.77	16.42	1.02
	7	24.27	16.60	1.07
2.5%	1	25.18	16.64	1.07
	2	24.42	17.15	1.09
	3	24.94	17.99	1.08
	4	24.28	17.10	1.07
	5	24.40	17.12	1.08
	6	24.24	16.69	1.10
	7	24.20	16.96	1.09
5%	1	24.16	17.80	1.12
	2	23.91	16.23	1.03
	3	23.98	16.37	1.07
	4	24.30	16.90	1.10
	5	24.11	16.58	1.09
	6	23.85	16.78	1.08
	7	23.94	16.53	1.05

Table 6 – Measures of Ufi Gel Hard specimens for surface free energy study.

% CHX loaded	Specimen Number	Measures (mm) Width	Height	Thickness
Control 0%	1	24.60	16.95	1.10
	2	24.71	17.28	1.03
	3	24.94	16.05	1.01
	4	24.29	16.92	1.07
	5	24.22	16.92	1.08
	6	25.05	16.66	1.03
	7	23.17	16.85	1.05
5%	1	24.18	17.27	1.06
	2	22.53	16.45	1.09
	3	24.95	16.47	1.11
	4	24.70	16.72	1.09
	5	24.19	16.66	1.07
	6	24.18	16.31	1.06
	7	24.62	16.36	1.06
7.5%	1	24.27	17.39	1.10
	2	24.27	17.07	1.06
	3	24.76	16.99	1.12
	4	24.71	16.40	1.12
	5	24.72	17.70	1.08
	6	24.17	16.24	1.10
	7	24.50	16.79	1.08

Table 7 – Measures of Probase Cold specimens for surface free energy study.

% CHX loaded	Specimen Number	Measures (mm) Width	Height	Thickness
Control 0%	1	25.08	16.49	1.02
	2	24.74	17.08	1.03
	3	24.90	16.36	1.03
	4	25.09	16.98	1.05
	5	25.13	17.62	1.04
	6	25.43	16.44	1.07
	7	24.70	17.06	1.04
2.5%	1	24.92	16.37	1.06
	2	24.22	17.20	1.08
	3	24.76	17.16	1.08
	4	24.90	16.08	1.02
	5	25.00	18.49	1.01
	6	24.33	16.86	1.03
	7	24.99	16.77	1.01
5%	1	24.58	16.43	1.01
	2	24.18	17.24	1.02
	3	24.59	17.74	1.07
	4	24.99	16.61	1.01
	5	24.36	16.67	1.09
	6	24.66	16.98	1.04
	7	24.83	17.15	1.08

Appendix 2 – Figures

Figure 1 – Kooliner.

Figure 2 – Ufi Gel Hard.

Figure 3 – Probase Cold.

Figure 4 – Probase Hot.

Figure 5 – Chlorhexidine Diacetate Monohydrate (CHX).

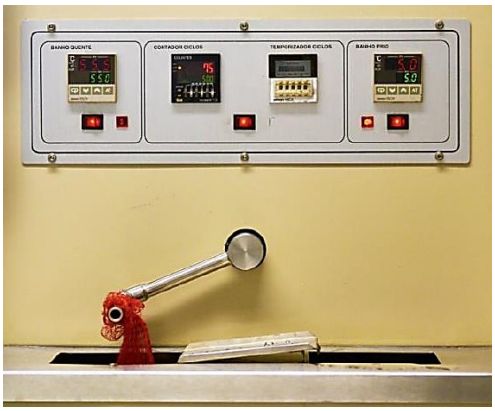

Figure 6 – Thermocycling equipment.

Figure 7 – Ivomat Pressure Device.

Figure 8 – Stereomicroscope: Equipment used in Failure mode assessment.

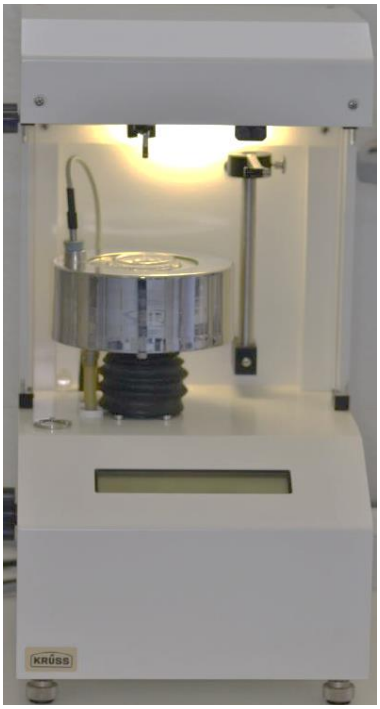

Figure 9 – Tensiometer K12: Equipment used in Wilhelmy plaque technique.

Figure 10 – Tensiometer K12: Equipment used in Wilhelmy plaque technique.

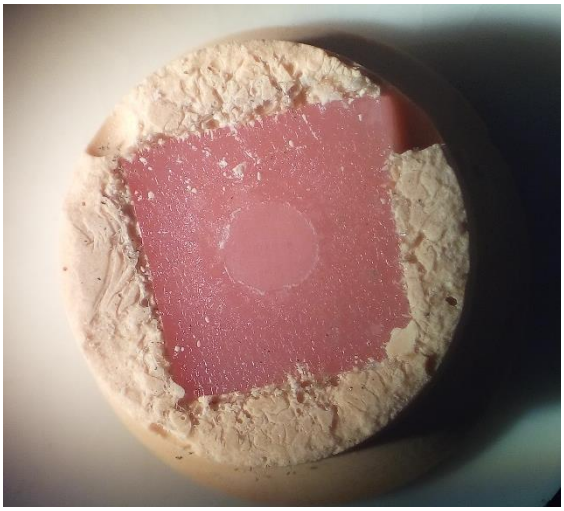

Figure 11 – Adhesive failure of a Kooliner specimen.

Figure 12 – Mixed failure of a Probase Cold Specimen.

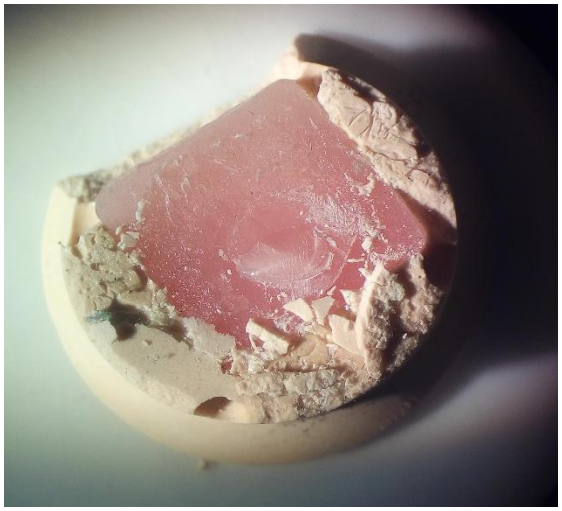

Figure 13 – Cohesive failure of a Probase Cold specimen.

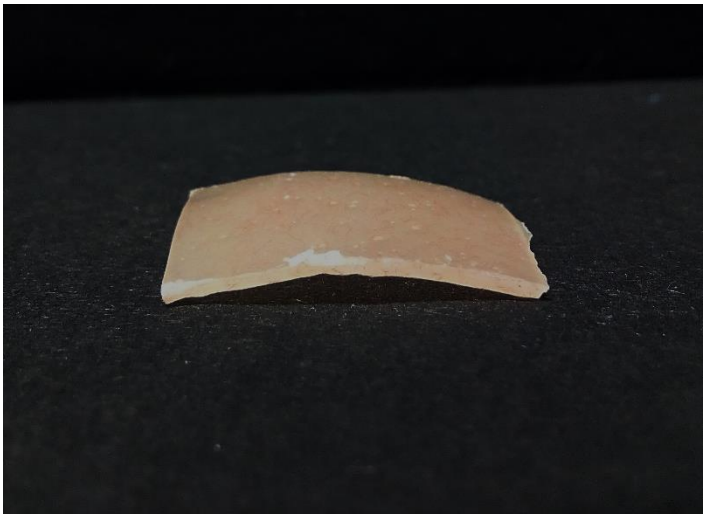

Figure 14 – Plastic deformation of a Kooliner specimen.

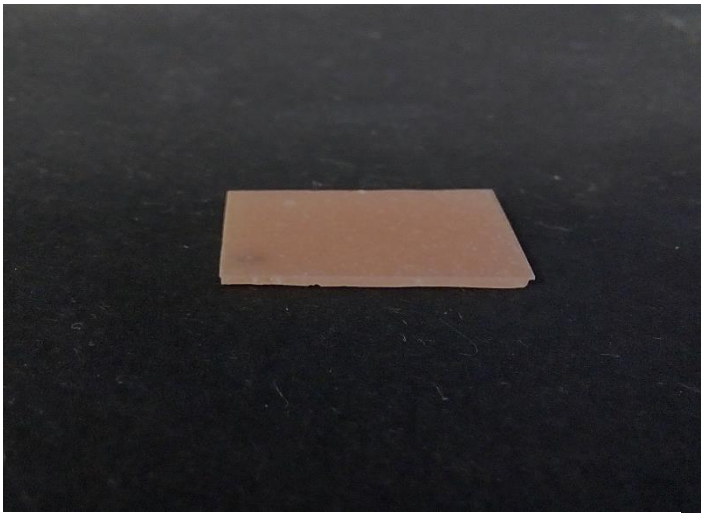

Figure 15 – Ufi Gel Hard specimen.

Appendix 3 – List of Tables

Appendix 4 – List of Figures

Appendix 5 – List of Abbreviations

1,6-HDMA	1,6-hexanedioldimethacrylate
CHX	Chlorhexidine
HEMA	2-hidroxyethylmethacrylate
IBMA	Isobutylmethacrylate
IR	Interquartile Range
K	Kooliner
L	Liquid
M	Mean
m	Median
Max	Maximum
Min	Minimum
min	Minutes
MMA	Methylmethacrylate
MPa	Megapascal
P	Powder
PC	Probase Cold
PEMA	Polyethylmethacrylate
PMMA	Polymethylmethacrylate
SD	Standard deviation
UF	Ufi Gel Hard
γ	Surface free energy
γ^d	Dispersive component of surface free energy
γ^p	Polar component of surface free energy

Appendix 6 – Experimental Data

1. Shear bond strength

1.1. Kooliner

% CHX loaded	Specimen Number	Shear Bond Strength (MPa)	Failure Mode
Control 0%	1	5.32	Adhesive
	2	13.40	Adhesive
	3	2.13	Adhesive
	4	5.55	Adhesive
	5	3.68	Adhesive
	6	3.63	Adhesive
	7	5.51	Adhesive
	8	4.53	Adhesive
	9	2.54	Adhesive
	10	7.69	Adhesive
2.5%	1	12.47	Adhesive
	2	8.70	Adhesive
	3	3.66	Adhesive
	4	4.24	Adhesive
	5	18.93	Adhesive
	6	7.58	Adhesive
	7	8.12	Adhesive
	8	7.99	Adhesive
	9	6.93	Adhesive
	10	4.14	Adhesive
5%	1	4.39	Adhesive
	2	3.49	Adhesive

	3	2.89	Adhesive
	4	2.70	Adhesive
	5	5.96	Adhesive
	6	8.08	Adhesive
	7	6.66	Adhesive
	8	4.33	Adhesive
	9	4.72	Adhesive
	10	3.60	Adhesive
7.5%	1	3.35	Adhesive
	2	5.15	Adhesive
	3	6.80	Adhesive
	4	2.61	Adhesive
	5	3.01	Adhesive
	6	4.81	Adhesive
	7	6.32	Adhesive
	8	5.53	Adhesive
	9	3.78	Adhesive
	10	9.69	Adhesive

1.2. Ufi Gel Hard

% CHX loaded	Specimen Number	Shear Bond Strength (MPa)	Failure Mode
Control **0%**	1	3.88	Adhesive
	2	10.72	Adhesive
	3	7.05	Adhesive
	4	17.61	Adhesive
	5	16.83	Adhesive
	6	7.18	Adhesive
	7	13.13	Adhesive

	8	7.50	Adhesive
	9	11.51	Adhesive
	10	2.40	Adhesive
2.5%	1	4.63	Adhesive
	2	7.21	Adhesive
	3	5.30	Adhesive
	4	4.79	Adhesive
	5	14.09	Adhesive
	6	5.01	Adhesive
	7	7.35	Adhesive
	8	5.20	Adhesive
	9	16.37	Adhesive
	10	7.91	Adhesive
5%	1	7.86	Adhesive
	2	11.15	Adhesive
	3	12.24	Adhesive
	4	10.23	Adhesive
	5	22.74	Mixed
	6	5.93	Adhesive
	7	11.34	Adhesive
	8	17.29	Mixed
	9	13.05	Mixed
	10	10.55	Adhesive
7.5%	1	10.85	Adhesive
	2	8.48	Mixed
	3	9.84	Adhesive
	4	4.25	Adhesive
	5	9.55	Adhesive
	6	8.94	Mixed
	7	10.45	Adhesive

	8	12.40	Adhesive
	9	7.94	Mixed
	10	11.14	Mixed
10%	1	29.97	Adhesive
	2	10.36	Mixed
	3	26.30	Adhesive
	4	5.87	Adhesive
	5	14.39	Adhesive
	6	14.36	Adhesive
	7	13.90	Mixed
	8	12.17	Adhesive
	9	25.01	Mixed
	10	1345	Mixed

1.3. Probase Cold

% CHX loaded	Specimen Number	Shear Bond Strength (MPa)	Failure Mode
Control **0%**	1	20.04	Cohesive
	2	29.24	Cohesive
	3	13.20	Adhesive
	4	34.46	Cohesive
	5	38.84	Cohesive
	6	27.54	Adhesive
	7	34.39	Mixed
	8	17.69	Mixed
	9	18.31	Adhesive
	10	25.57	Mixed
2.5%	1	28.31	Mixed
	2	15.87	Mixed

	3	14.59	Adhesive
	4	16.43	Adhesive
	5	18.74	Mixed
	6	9.32	Cohesive
	7	11.73	Adhesive
	8	22.20	Mixed
	9	18.50	Adhesive
	10	17.47	Adhesive
5%	1	8.85	Adhesive
	2	11.36	Adhesive
	3	19.21	Adhesive
	4	15.79	Mixed
	5	15.34	Adhesive
	6	21.53	Mixed
	7	10.56	Mixed
	8	21.70	Adhesive
	9	23.01	Adhesive
	10	14.73	Mixed

2. Surface free energy

2.1. Kooliner

% CHX loaded	Specimen Number	Advance Contact Angle (°)		Surface Free Energy (γ) (mN/m)		
		Water	1,2-Propanediol	γ Total	γ Dispersive	γ Polar
Control 0%	1	78.40	101.52	41.6	0.0	41.7
	2	84.07	69.92	27.9	8.3	19.6
	3	87.37	54.11	26.9	14.9	12.0
	4	79.08	59.25	30.8	11.1	19.7
	5	90.67	62.13	24.5	12.4	12.0
	6	85.98	62.03	26.7	11.3	15.4
	7	77.63	59.62	31.7	10.7	210
2.5%	1	86.89	55.05	27.0	14.3	12.6
	2	85.66	75.24	27.2	6.8	20.4
	3	90.39	65.90	24.3	10.8	13.4
	4	87.41	91.34	28.5	2.6	25.9
	5	87.77	61.08	25.9	12.1	13.8
	6	84.51	63.27	27.5	10.6	16.9
	7	88.84	63.69	25.1	11.2	13.9
5%	1	79.72	60.64	30.4	10.7	19.7
	2	91.68	65.62	23.7	11.2	12.4
	3	85.22	65.85	27.0	9.8	17.2
	4	87.26	65.01	25.9	10.5	15.4
	5	90.85	66.70	24.0	10.6	13.4
	6	86.17	64.15	26.5	10.6	15.9
	7	78.67	57.01	31.1	11.8	19.3

2.2. Ufi Gel Hard

% CHX loaded	Specimen Number	Advance Contact Angle (°)		Surface Free Energy (γ) (mN/m)		
		Water	1,2-Propanediol	γ Total	γ Dispersive	γ Polar
Control 0%	1	92.90	75.58	22.7	7.8	14.9
	2	90.88	66.70	24.0	10.6	13.3
	3	93.50	44.76	28.5	22.9	5.7
	4	82.84	57.59	28.7	12.4	16.3
	5	75.54	73.41	34.7	6.3	28.4
	6	92.55	82.22	23.2	5.6	17.6
	7	74.63	71.77	35.2	67	28.5
5%	1	75.25	51.40	33.3	13.2	20.2
	2	75.49	100.67	44.4	0.0	44.4
	3	88.93	50.84	27.0	16.9	10.1
	4	86.91	78.36	26.6	6.0	20.6
	5	92.12	54.89	33.3	12.0	21.4
	6	75.13	59.37	32.0	10.8	21.2
	7	77.24	67.67	23.2	10.5	12.8
7.5%	1	81.00	48.93	30.4	15.2	15.1
	2	82.54	68.94	28.8	8.4	20.5
	3	79.02	54.96	30.9	12.6	18.4
	4	80.54	82.90	32.4	4.1	28.2
	5	85.34	58.80	27.3	12.4	14.8
	6	83.14	58.81	28.4	12.0	16.5
	7	90.64	63.09	24.4	12.0	12.4

2.3. Probase Cold

% CHX loaded	Specimen Number	Advance Contact Angle (°)		Surface Free Energy (γ) (mN/m)		
		Water	1,2-Propanediol	γ Total	γ Dispersive	γ Polar
Control 0%	1	82.40	73.72	29.3	6.9	22.5
	2	87.16	78.37	26.4	6.0	20.4
	3	86.21	62.80	26.6	11.1	15.5
	4	90.64	93.73	26.3	2.3	24.1
	5	85.95	59.99	26.9	12.1	14.8
	6	87.43	77.07	26.1	6.5	19.7
	7	88.75	56.12	26.1	14.4	11.6
2.5%	1	84.87	67.88	27.3	9.1	18.2
	2	87.31	75.57	26.1	6.9	19.2
	3	86.65	84.69	27.7	4.2	23.5
	4	81.22	58.34	29.5	11.8	17.8
	5	88.43	66.67	25.2	10.1	15.1
	6	85.03	87.18	29.5	3.4	26.1
	7	81.58	59.06	29.3	11.6	17.7
5%	1	79.66	64.31	30.5	9.5	21.1
	2	78.60	61.13	31.1	10.4	20.8
	3	92.93	63.25	23.4	12.6	10.8
	4	81.95	66.35	29.1	9.1	20,0
	5	95.50	63.43	22.5	13.4	9.1
	6	78.73	61.21	31.1	10.4	20.7
	7	83.30	60.72	28.3	11.3	17.0

Appendix 7 - Graphs

Surface free energy

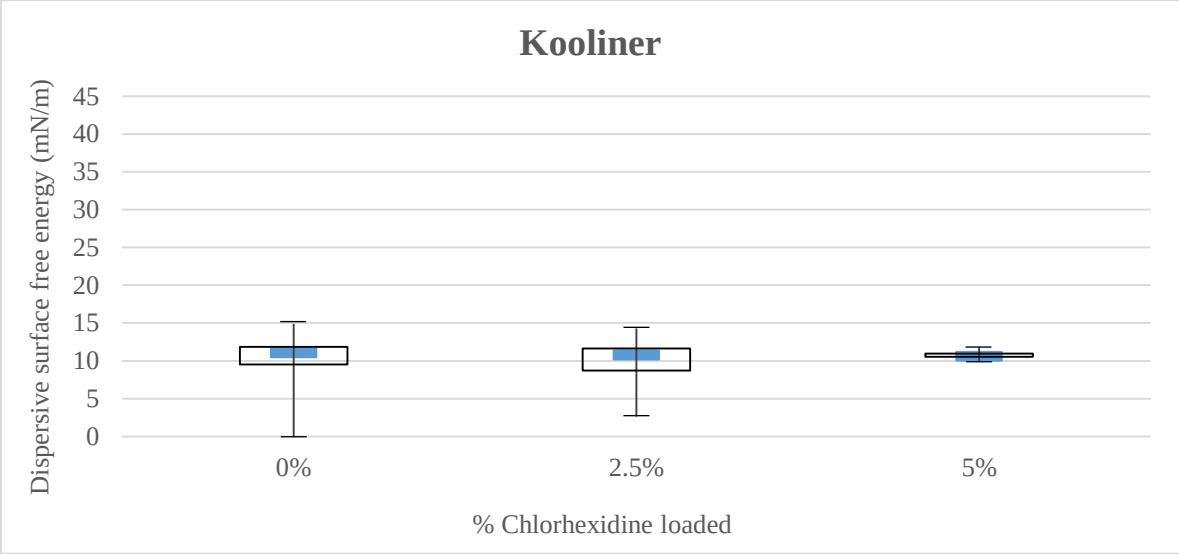

Figure 16 – Median and Interquartile Range of Kooliner for dispersive surface free energy.

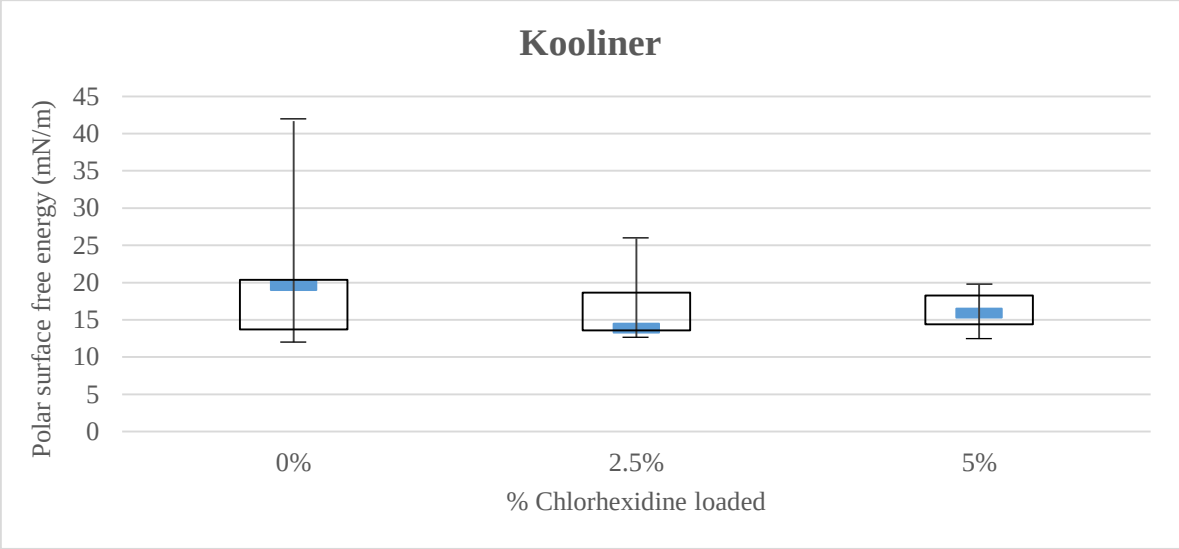

Figure 17 – Median and Interquartile Range of Kooliner for polar surface free energy.

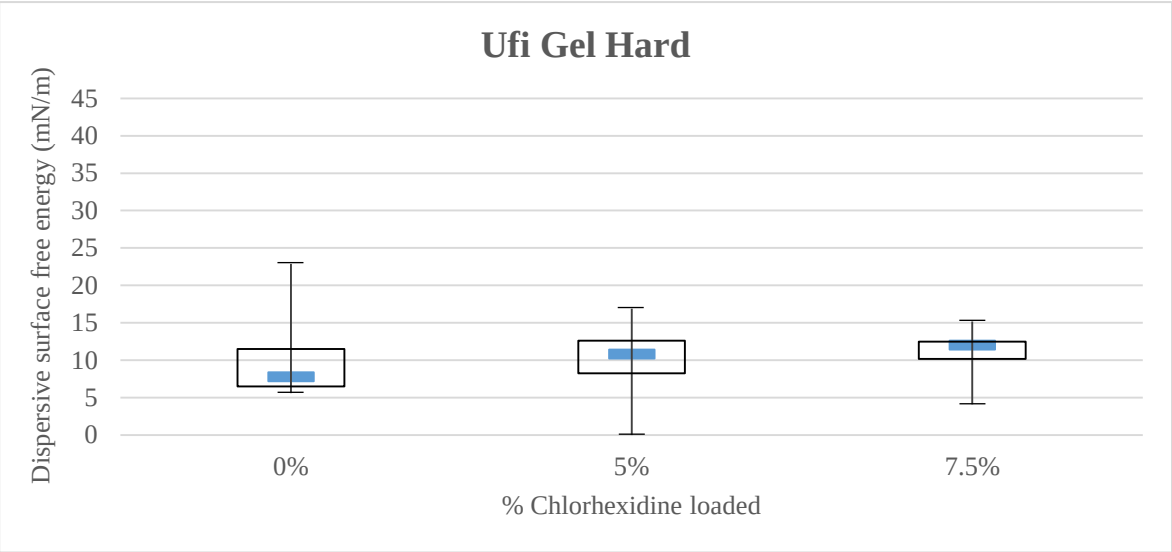

Figure 18 – Median and Interquartile Range of Ufi Gel Hard for dispersive surface free energy.

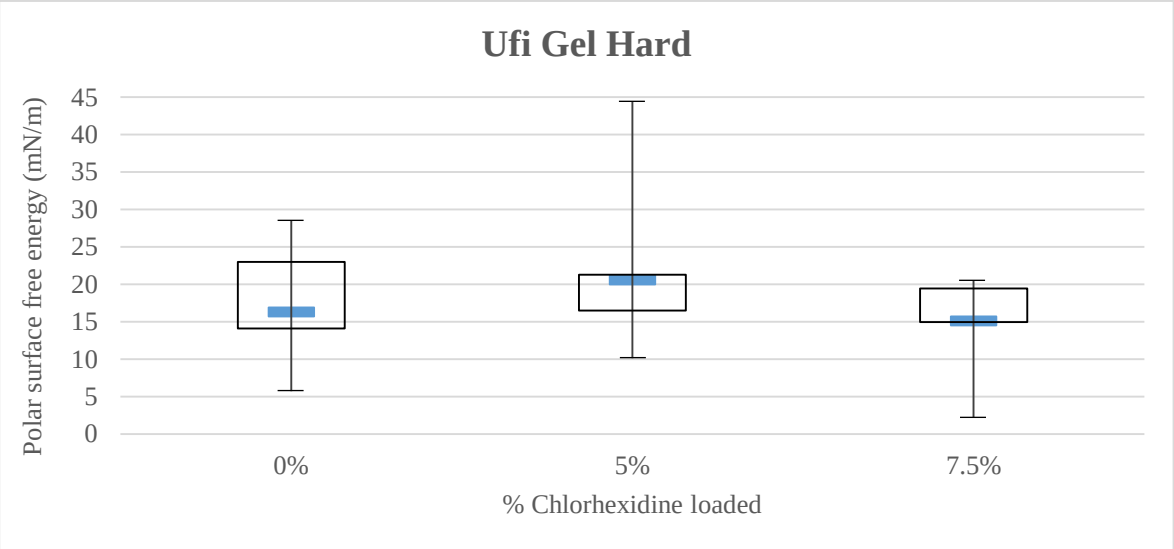

Figure 19 – Median and Interquartile Range of Ufi Gel Hard for polar surface free energy.

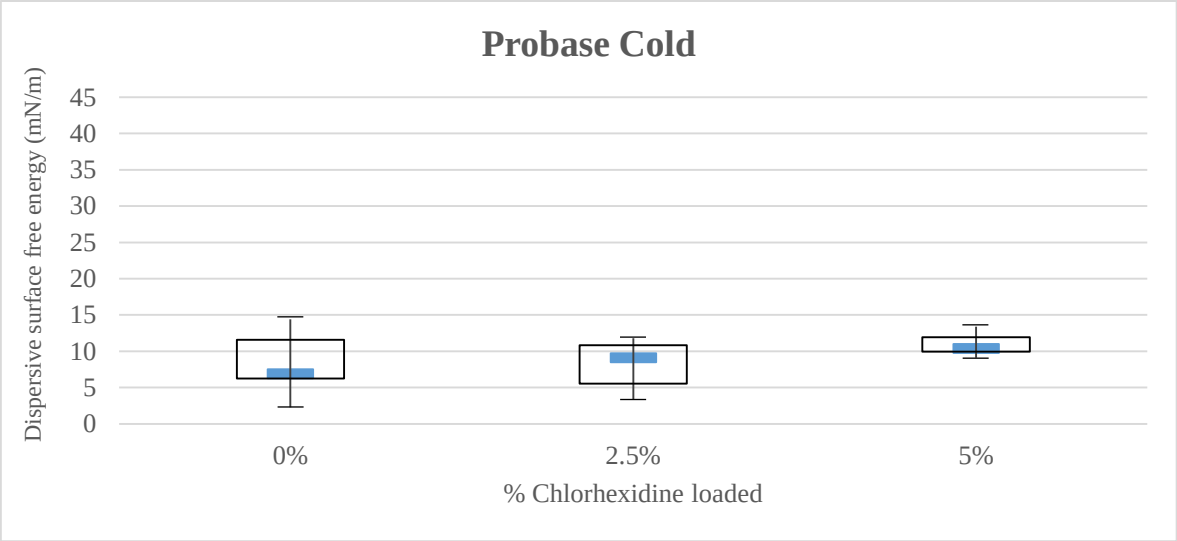

Figure 20 – Median and Interquartile Range of Probase Cold for dispersive surface free energy.

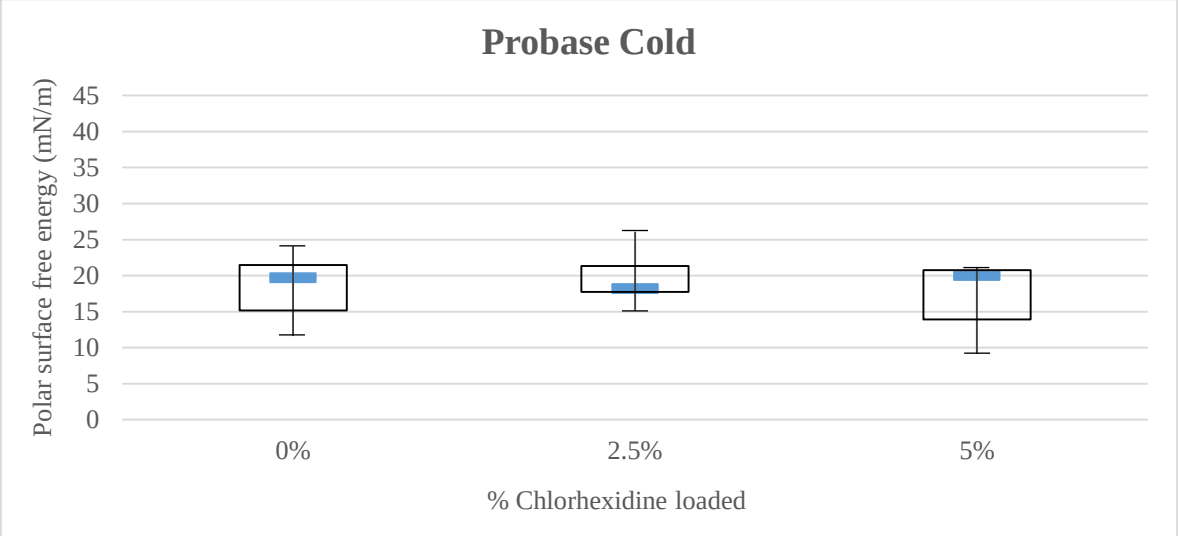

Figure 21 – Median and Interquartile Range of Probase Cold for polar surface free energy.